Volatile Conversations

How Barley cultivars shape each other through VOC-mediated phenotypic and genetic responses

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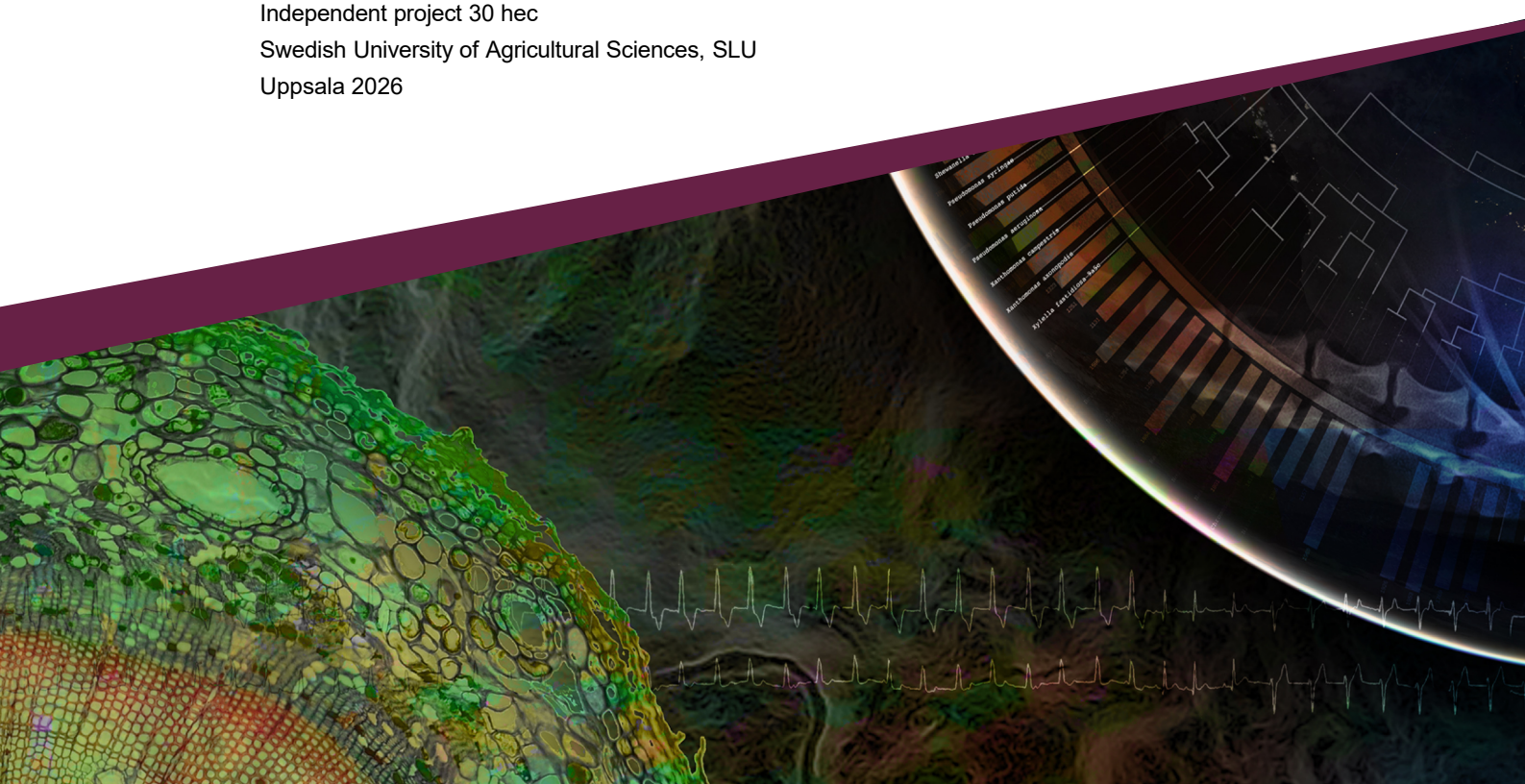

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Abstract

This study examines volatile-mediated interactions among three barley cultivars Salome, Fairytale and Luhkas when grown under non-stressed conditions. Using a two-chamber exposure system to isolate airborne interaction from other environmental factors, we assessed phenotypic responses and differential gene expression in the receiver cultivar Salome after exposed to volatiles emitted by either Salome (self-exposure control), Fairytale, or Luhkas.
Salome reacted strongly to volatiles from the slow-growing cultivar Fairytale, characterized by reduced growth and significant upregulation of defence- and stress-related genes. In contrast, exposure to volatiles from Luhkas caused no measurable changes in either growth or gene expression. These contrasting outcomes demonstrate that volatile-mediated interactions can promote phenotypic convergence, especially when initial growth rates between emitter and receiver cultivars differ substantially.

Keywords: Volatile Organic Compounds, Barley, Plant interactions, Crop Resilience, Increased Similarity, Mimicking, Differential gene expression, Growth Strategies, Competition

Popular Science Summary

Plants are often seen as passive organisms yet there is constant communication taking place. Conversations we might have picked up without understanding the message. Have you ever smelled newly cut grass? Is so, you have already sensed one way of plant-plant communication which happens through Volatile Organic Compounds (VOCs). We are not the only ones who can smell the grass, surrounding plants can “smell” it too.

This study examines how different types of Barley influence each other through such airborne signals. Three cultivars with different growth characteristics were selected: the fast growing and relatively large Salome, the slow growing and smaller Fairytale and Lukhas, which was average. By isolating these plants in plastic cages, where the only form of interaction possible was through airborne signals, we were able to specifically examine the effects of VOC-mediated interactions.

Results shows that when Salome scented VOCs from the slower growing Fairytale, it reduced its growth and altered the expression of several genes associated with defence and stress. This suggests that Salome adapts and becomes more similar to Fairytale, a phenomenon known as phenotypic convergence. By reducing its growth, Salome may avoid unnecessary competition while simultaneously preparing for future challenges.

In contrast, Salome showed no clear response to VOCs emitted by Luhkas. Since these two cultivars are already more similar, the need for further adaptation may be reduced. This indicates that the degree of initial difference between plants may play a greater role in determining their response than the identity of the neighbouring plant itself. However, further studies including a wider range of cultivars are needed to confirm this pattern.

The study also demonstrates that plant responses are not only visible in their physical appearance but also occur at the molecular level. Changes in gene expression represent an early response to environmental cues, allowing plants to adjust their strategies before direct competition for resources arises. These findings are important for future agriculture. They show that natural interactions between plants can influence growth, resilience, and stress responses. If utilized effectively, such interactions could help reduce the need for fertilizers, pesticides, and other chemical inputs. This study highlights how these quiet “conversations” between plants could be harnessed to develop more sustainable agricultural systems.

Table of contents

List of Figures

List of Tables

Abbreviations

VOC	Volatile Organic Compounds
LMF	Leaf mass fraction = Leaf dry mass / total plant dry mass
RMF	Root mass fraction = Root dry mass / total plant dry mass
SRR	Shoot-Root ratio = Leaf dry mass / total plant dry mass
SLA	Specific Leaf Area = Leaf Area / Leaf dry mass
SEFt	Salome exposed to Fairytale.
SEL	Salome exposed to Luhkas
SES	Salome exposed to Salome
S	Salome
Ft	Fairytale

1. Introduction

Diversity in modern agriculture is declining and only a few species dominating current production systems. The challenge of feeding a rapidly growing population has driven immense changes in agricultural landscape and to reduce the risks of unwanted long-term consequences, it is essential to promote sustainable food production. Crop species mostly consists of several different genotypes and growing them in mixtures is one of the solutions to increase diversity in production of single species while maintaining a higher yield. Cultivar mixtures refer to the simultaneous cultivation of two or more cultivars of the same species within the same field. This approach increases intraspecific diversity while maintaining uniform management practices. In contrast, crop mixtures comprise combinations of different species, and when these are grown in spatial and temporal proximity with deliberate functional complementarity, the system is referred to as intercropping. These diversity-based strategies can enhance resource-use efficiency and improve overall crop performance. Greater diversity can improve resilience to pest and, and environmental stressors (Borg et al., 2018; Reiss & Drinkwater, 2018). Monocultures, by contrast, often lead to ecosystem imbalance that must be compensated for through substantial fertilizer inputs. However, by combining compatible crops, the nutrient uptake efficiency could be increased (Li et al., 2018) while fertilizers may have the opposite effect. Excess fertilizer application elevates the risk of nutrient leakage and subsequent environmental pollution (Huchelmann et al., 2017). We are facing an increasing trend of global demand when it comes to fertilizers. Instead of using more plant nutrition, we should focus in improving the nutrient uptake ability within the plants (Salim & Raza, 2020). Therefore, increasing diversity through cultivar mixtures could provide more than sustainable agriculture, it also offers an efficient, resilient and environmentally friendly food production.

Different outcomes depending on what cultivar types are grown together

Plants interact in many ways, but the outcomes of these interactions vary considerably on the specific cultivar pairing. Some cultivars show functional complementarity, minimizing competition by exploiting different ecological niches and accessing distinct pools of resources. This complementary resource use enhances overall efficiency of resource, leading to facilitation and overyielding (Connolly et al., 1990; Hauggaard-Nielsen et al., 2001). However, several studies have reported that field-level diversification does not always result in favourable trade-off under standard farming conditions (Mansion-Vaquié et al., 2019; Reiss & Drinkwater, 2018). This indicates that mixtures can be highly beneficial in some cases but not in others, highlighting the importance of selecting compatible cultivar combinations. The magnitude and direction of mixture effects vary depending on which cultivars are combined and some cultivars showing strong positive interactions than others (Borg et al., 2018; Loreau & Hector, 2001; Ninkovic et al., 2016). Such variability highlights the inherent complexity of plant–plant interactions. This complexity underlines the need for research aimed at identifying optimal cultivar pairs to support sustainable agricultural practices.

Evolutionary strategies in response to environmental cues

Allelopathy have demonstrated several direct effects on growth responses in plants (Åbonde, 2025; Dahlin, 2019; Ninkovic, 2003). Altered growth patterns are often part of a greater survival strategy and there are several theories, explaining the evolutionary reason for different growth responses.

Phenotypic convergence for coexistence

Plants growing in close proximity engage interact through diverse mechanisms, ranging from competition and facilitation to various forms of biochemical communication. One notable signalling pathway involves the emission and perception of airborne volatile organic compounds (VOCs). Although interest in VOC-mediated plant–plant interactions is increasing, our understanding of how these cues influence growth and gene expression under non-stressed conditions remains limited. Volatile interactions enable coexistence in greater plant communities. It assists in coordination with surrounding plants and synchronization in flowering and defence. Cultivars growing together often evolve similar phenotypes due to exposure to the volatile profiles of each other and competition-derived niche reinforcement (Dahlin et al., 2019; Srikanth et al., 2024). Adaptive similarity can be observed in phenotype when VOCs from stressed emitters induce defence-mechanisms and upregulated stress also in receivers (Markovic et al., 2019; Ninkovic et al., 2021). Few studies have investigated how VOCs impact adaptive similarity of plants growing in non-stressed conditions. This has been performed in field experiments, but the phenotype of a cultivar is regulated by many factors and increased similarity can also be a result of exposure to similar environmental threats when growing openly in the field (Dahlin et al., 2019).

Competition under resource limitation

Competition is generally defined as a negative interaction between individuals with overlapping niches which share the same physical space. It arises when the availability of resources jointly required by both parties becomes limited (Gillet, 2008). Consequently, the intensity of competition typically increase with great similarity between interacting individuals (Holden & Cahill, 2024). The distinct volatile profile produced by one cultivar can influence the development of another, allowing them to adjust their competitive strategies to ensure access to essential resources (Brilli et al., 2019; Ninkovic et al., 2016). However, plants with comparable competitive abilities do not always outcompete one other, instead they may coexist while relying on shared resources pool (Holden & Cahill, 2024). The ability to perceive VOCs and identify neighbours with overlapping niches allows plants to prime themselves for potential future competition. As a result, competitive traits can emerge even before direct resources competition occurs, and such early responses are detectable at level of genes (Ninkovic et al., 2016).

Kin Selection and Altruism

The kin selection theory suggests that individuals may behave altruistically toward to genetically related conspecifics, and kin recognition refers to ability of plant to distinguish relatives from non-relatives (Li et al., 2018). However, kin recognition is a species-specific trait and has only been observed in a limited number of plant species. Several studies report reduced competition among kin, suggesting the presence of kin-biased interactions (Lim et al., 2020; Ninkovic, 2003; Spinelli et al., 2011). Kin recognition presumes that each genotype or species produces a distinct volatile profile and that related individuals are more sensitive to these signals than to volatiles emitted by distantly related species. Such recognition can reduce competitive responses among kin and promote coordinated defense strategies, resulting in enhanced resistance against pests (Brilli et al., 2019; Lim et al., 2020; Spinelli et

al., 2011). Such cooperative effects may ultimately enhance offspring fitness and increase survival (Heil & Karban, 2010). Kin recognition might not be required for kin selection, because neighbours plants are often genetically related and only nearby individuals are exposed to, and benefit from emitted VOCs (Dudley et al., 2013; Heil & Karban, 2010). Intraspecific interactions, common in stands dominated by a single species, can therefore create conditions under which kin selection can arise (Lepik et al., 2012).

Interaction through Volatile Organic Compounds

When different cultivars grow in proximity, they are continuously exposed to each other's VOCs. VOC emission is a functional plant traits, but both the composition and intensity of emitted volatiles vary depending on environmental conditions, plant developmental stage or stress level (Ninkovic et al., 2020). VOCs contain fingerprint information about genetic identity and status of the emitting plant, enabling neighbouring individuals to eavesdrop on potential competitors and their growth or defence strategies accordingly (Ninkovic et al., 2016, 2020). This incurs a non-benefitable metabolic cost for the emitter in interspecific interactions. Therefore, it's questionable whether "plant-plant communication" is the real reason for VOC emission (Arimura et al., 2010).

Evaluation of responses through differential gene expression

Responses to environmental cues can often be detected through phenotypic changes, however, many reactions occur at a molecular level and cannot be captured through phenotypic analysis alone. All such responses originate from alterations in gene expression which can be quantified using a range of molecular techniques. Some of these methods include high throughput DNA or RNA sequencing that are appropriate for targeting greater number of genes (Avison, 2008). Data generated from RNA sequencing can be analysed through differential gene expression analysis to identify significant differences in amount of RNA across multiple sets of samples and treatments (Rosati et al., 2024). In barley, the first barley reference genome (IBSC V1) was constructed in 2012 by shotgun sequencing (Mayer et al., 2016). The reference genome was further improved in 2017 when IBSC V2 was assembled through Illumina-sequencing, resulting in increased genome completeness and accuracy (Xu et al., 2021).

Barley

As one of the world's most important cereal crop, barley is cultivated across many countries and has a worldwide distribution. It is a highly adaptable crop due to its high tolerance to abiotic and biotic stress, as well as its diverse range of end uses (Geng et al., 2022). Growing winter barley in mixtures instead of monocultures have shown to result in higher and more stable yields across seasons (Creissen et al., 2016). Barley generally shows increased adaptive similarity when grown in mixtures compared with when grown in pure strands. But different barley cultivar respond differently depending on their combination (Ninkovic et al., 2019). The responses vary not only across on strains but also depending on individual plant within the same family. Experiments performed on different barley cultivars have reported diverse responses depending on cultivar combinations. VOCs mediated interactions have been shown impact on plant biomass allocation, nutrient uptake efficiency, adaptive similarity and biomass production and aphid feeding behaviour, and their growth development, (Åbonde, 2025; Dahlin, 2019; Dahlin et al., 2018; Kheam et al., 2023; Ninkovic, 2003; Ninkovic et al., 2002, 2016). Adaptive similarity has also been observed in studies of aphid resistance in cultivar mixtures. When exposed to volatiles from resistant emitter the receiver plant can mimic these defensive traits and thereby increases its own resistance to aphids (Ninkovic & Åhman, n.d.; Tooker & Frank, 2012).

While VOCs have been extensively studied in the context of plant stress responses and defence signalling, their roles in regulating plant development under non-stressed conditions remain less understood. This thesis addresses this knowledge gap by investigating how VOCs emitted by different barley cultivars affect both the growth and gene expression in a receiving cultivar. By focusing on controlled, non-stressed environments, this study aims to clarify how cultivar-specific volatile cues contribute and drive phenotypic adjustment and molecular responses. These findings provide new insights into the mechanisms underlying cultivar compatibility, adaptive similarity, and the potential benefits of cultivar mixtures in agricultural systems.

2. Scope

This study aims to investigate the impact of volatile mediated interactions among different barley cultivars growing under non-stressed conditions. Previous research has shown that the cultivars Fairytale and Luhkas can stimulate pronounced responses in Salome (Dahlin et al., 2018) which motivated the selection of these cultivars for the present work. This thesis specifically examines how a fast-growing cultivar like Salome responds to VOC cues emitted by slower growing cultivars Fairytale and intermediate-growing Luhkas, and explores potential growth strategies that may be adopted depending on the identity of the emitter.

Volatile inter-plant communication:
This study examines the potential of VOCs to adjust growth and gene expression in the neighbours in the absence of any external influences such as nutrient competition or herbivory.

Alterations in phenotype and gene expression.
It investigates how changes in gene expression relate to phenotypic growth traits, including root development, plant height and biomass allocation.

Responses that are cultivar specific
This study examines cultivar-specific responses to VOCs exposure by testing whether emission from slow-growing barley cultivar Fairytale and the intermediate-growing cultivar Luhkas alter the growth response of the fast-growing receiver cultivar Salome.

Regulated conditions:
The present study is conducted under controlled environmental conditions that isolated VOC-mediated effects from other environmental impacts. This methodological approach ensures that the observed responses can be attributed exclusively to airborne signalling rather than to confounding environmental factors.

Agricultural significance:
The results of this study indicate that cultivar mixtures can influence plant competition, resilience and growth dynamics underscoring their potential to support more sustainable agriculture systems.

3. Hypotheses

I. *We hypothesize that the exposure of the fast-growing barley cultivar Salome to volatile organic compounds (VOCs) emitted by the slower-growing cultivars Fairytale and Luhkas suppresses its growth, thereby reducing phenotypic differences and promoting convergence in growth traits between the emitter and receiver cultivars.*

II. *These phenotypic changes are expected to be accompanied by distinct differential gene expression profiles, reflecting the underlying molecular responses triggered by VOC exposure.*

III. *A positive correlation is expected between the magnitude of initial phenotypic differences among cultivars and the extent of their convergence in response to VOC exposure.*

4. Material and Method

The spring barley (*Hordeum vulgare* L.) cultivars Fairytale, Salome, and Luhkas (Scandinavian Seeds AB, Sweden) were selected based on their contrasting growth dynamics, previously characterized by (Dahlin et al., 2020) as exhibiting slow, intermediate, and rapid growth rates, respectively.

Experimental design

The experiment was conducted in a plant exposure system (Ninkovic, 2003) to isolate the effects of VOCs from mean of interaction between plants such as root exudates or physical contact. Plastic tubes made of cylindrical polyethylene (2 x 85 cm) with arrogation holes were filled with sand (Silversand 55, Sibelco Europe, Mölndal, Sweden) and attached to table though the hole (5x2 cm). A black plastic curtain was fixed around the bench to keep in complete darkness. Any gaps in the hole were sealed with sponge to prevent light penetration to the roots. Two days before sowing, each tube was connected to a drip-irrigation system to ensure adequate sand moisture for plant growth. The seeds of three barley cultivars (Fairytale, Luhkas & Salome) were sprouted overnight in petri dishes on moist filter paper. One germinated seed was planted at 1 cm depth and carefully covered with dry sand. Four days after sowing, transparent twin-Perspeks chambers were placed over the plants (*Figure* 1). A vacuum tank provided airflow from emitter (in the left cage) to receiver (in the right cage).

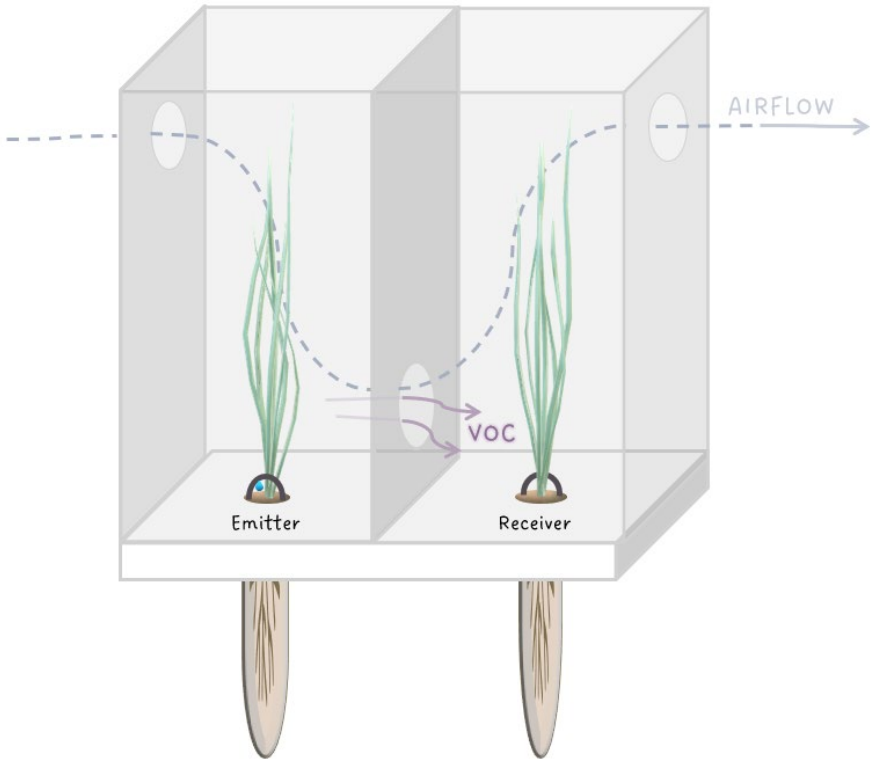

Figure 1: ***Experimental setup showing the two chambers****: one for the emitter (at the left side) and one for the receiver (at the right side). Air was drawn out through the hole in the upper right corner creating air flow causing emitted volatiles to travel from the emitter in the left chamber to the receiver in the right chamber. Roots grew in separate bags, hanging under the table and an automatic water system was connected to each plant.*

Irrigation System

An irrigation system powered by a pump provided water to each plant via thin tubes. The system kept the soil uniformly moist during first six-days after planting, the system ran on a 15 min on/off cycle: 15 minutes of irrigation followed by 15 minutes without water (*Appendix: Table 4*). This intermittent regime was used to prevent preferential flow along a single capillary pathway and to promote even water distribution throughout the sand substrate. After 6 days when plants reached the first leaf stage, Blomstra plant nutrition (*Appendix: Table 3*) was added to the irrigation system (2 ml/l) and irrigation schedule was changed. The system then operated for 15 minutes, with 30 minutes interval between cycles during daytime and 60 minute interval between cycles at night (*Appendix: Table 3*). Supply of nutrition was gradually reduced during the experiment.

Growth conditions

Plants were grown in a climate-chamber under temperature of 20±2 ºC and humidity 60 with light regime of 16 hours of light and 8 hours of darkness. Light was supplied by Light4Food lamps mounted 1 m above the table. The setup consisted of a central light strip (Philips LED, 77 W, Poland) and two side lights positioned to the left and right (Philips LED lighting IBRS, 55–60 W, Netherlands), each operating under its own lighting regimen. The LED light had 28% blue, 38% green and 34% red light (*Appendix: Table 2).*

Replicates

The three following combinations were established with 12 replicates: Salome exposed to volatiles from Fairytale (SEFt); Salome exposed to volatiles from Luhkas (SEL); and Salome exposed to volatiles from Salome (SES). Plants were divided into blocks where each block included one of each cultivar pairing SEL, SEFt and SES to minimize potential environmental gradients or positional effects (*Figure* 2). The experiment was conducted in two runs with 12 replicates per combination.

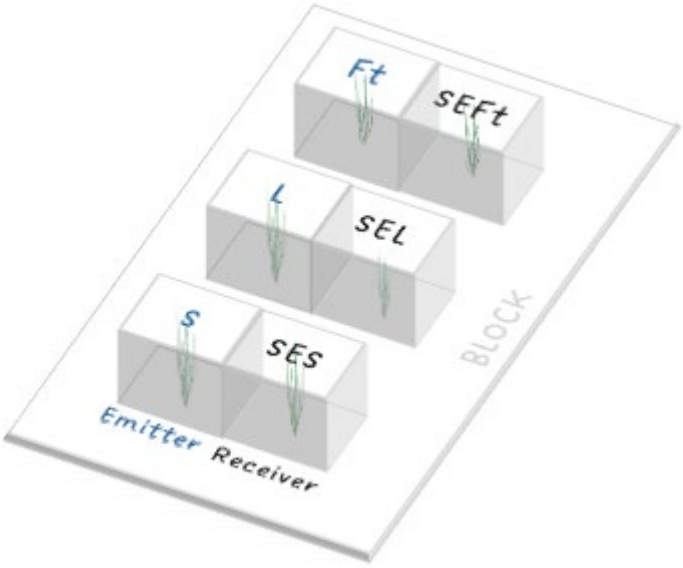

Figure 2: ***Setup of treatments in one block****. Emitters (blue), receivers (black) and non-exposed (green).* ***S****= Salome,* ***SES****= Salome exposed to Salome,* ***L****= Luhkas,* ***SEL****= Salome exposed to Luhkas,* ***Ft****= Fairytale,* ***SEFt****= Salome exposed to Fairytale.*

Collection of plant material for sequencing

Before harvesting entire plant (25 days after sowing), 1 cm from the tip of the first leaf was sampled and promptly transferred into liquid nitrogen and then stored in the freezer at -80°C. RNA was extracted from the samples through whole RNA purification according to the TRIzol (Invitrogen) protocol (Life Technologies). To obtain mRNA for RNA sequencing, samples were purified with the NEB mRNA isolation kit (New England Biolabs, Ipswich, MA, USA). The purified mRNA was used to prepare RNA high-throughput libraries with the NEBNext Ultra II Directional Library preparation kit for Illumina (New England Biolabs, Ipswich, MA, USA).

The libraries were marked with individual barcodes and sequenced in one lane of an Illumina HiSeq 2500.

Sampling

25 days after sowing, all plants, both emitters and receivers were separated into three components: leaves, stems, and roots. The root systems were carefully excavated, thoroughly rinsed in bucket half filled with water to remove adhering sand particles, and subsequently stored in plastic bottles filled with 10% ethanol solution for preservation. The aboveground portion of each plant was measured to determine shoot height. The same procedure was applied to stems. Prior to scanning, roots were removed from plastic bottle and placed in a Petri dish filled with distilled water to prevent desiccation and maintain structural integrity. Roots were scanned in a transparent Plexiglas box filled with distil water. Following image acquisition, all plant components: roots, stems, and leaves were oven-dried at 70 °C for 48 hours. Dried samples were then left to equilibrate at room temperature for 24 hours before being weighed using a high-precision analytical balance.

Figure 3: ***Leaves scanned in WinRHIZO***

Analysis

Phenotypic Analysis

Barley is a staple crop containing several cultivar types with different phenotypic traits. Salome is a cultivar type that generally have greater specific leaf area (SLA) while Luhkas identifies as a fast-growing type of barley, producing large plants. Fairytale is slow growing and produce smaller leaves (Dahlin, 2019).

Length and area of the different plant parts were analysed using the digital image analysis software WinRHIZO, The leaf mass fraction (LMF) was calculated as the ratio of dry leaf mass to total plant dry mass, indicating the proportion of biomass allocated to leaf development. This metric reflects the plant's investment in leaf growth relative to other parts. Similarly, the root mass fraction (RMF) was calculated as dry root mass divided by the total plant dry mass, and the stem mass fraction (SMF), was calculated as dry stem mass divided by the total plant dry mass. The total dry weight (TDW) was determined as the sum of the dry weight of all plant parts (leaves, stem, and roots). The shoot-root ratio (SR) was defined as the proportion of leaf and stem dry mass to root mass. This ratio provides an indication of biomass allocation between above-ground and below-ground plant biomass. A high SR shows a greater allocation of biomass to shoots relative to roots. The specific leaf area (SLA) was calculated to assess the leaf surface area relative to leaf dry mass, reflecting the area of area of leaves of leaf produced per gram of dry mass (*Table 1*). Statistical analysis was performed using the software "STATISTICA" and exported raw data was plotted in excel with error bars illustrating standard error. To define significant differences between treatments with confidence level at 95%, a two-way ANOVA was performed with treatment and block as two fixed factors. It was followed by a Tukey's HSD post-hoc test to identify significant differences ($p \leq 0.05$) between the treatments.

Category	Trait	Description	Unit
Biomass	Total plant biomass	Dry weight of total plant	g
	Above ground biomass	Dry weight of stem and leaves combined	g
	Leaf mass	Dry weight of total biomass in leaves	g
	Stem mass	Dry weight of total biomass in stem	g
	Root mass	Dry weight of total biomass in roots	g
Elongation	Plant height	Total plant height	cm
	Stem height	Height of the main stem	cm
	Root Length	Total length of roots	cm
	Leaf surface	Total leaf surface of all leaves	cm^2
	Average root diameter	Mean diameter of roots	mm
Biomass Allocation	Shoot-root ratio (SRR)	Proportion of biomass allocated to above ground plant parts	N/A
	Stem-mass fraction (SMF)	Proportion of biomass allocated to stem	N/A
	Leaf-mass fraction (LMF)	Proportion of biomass allocated to leaf	N/A
	Root-mass fraction (RMF)	Proportion of biomass allocated to roots	N/A
	Specific leaf area (SLA)	Leaf area per unit leaf dry mass	cm^2/g

Table 1: ***Calculated growth-parameters for phenotypic analysis.***

Sequencing analysis

Quality control was performed on the Illumina sequencing output data and low quality reads were removed. The remaining reads were adapter trimmed, de-multiplexed and filtered according to length. RNA sequencing paired reads were aligned to the barley genome (IBSC, version 2) using bowtie2 (Langmead & Salzberg, 2012) with default parameters. Reads per gene were counted using HTSeq-counts (Anders et al., 2014), parameters -mode union -stranded no -minequal 10 and -nonunique none, resulting in count tables which could be used in DESeq2 (Love et al., 2014) to conclude significant expression with fit type set to parametric. All analysis tools were used through the Galaxy platform (Afgan et al., 2018) Genetic networks were identified through BarleyNet pathway-centric search tool (http://www.inetbio.org/barleynet/search.php) (Lee et al., n.d.)

Statistical analysis

The Mixed procedure of the SAS package was used for the analyses of morphological parameters where block was set as fixed and treatments*block was set as random effect. Diagnostic plots were prepared to evaluate the experimental theory and Turkey´s HSD test was used for calculation and comparison of least squares means. The expression level of genes and transcripts was quantified by counting aligned reads and differences. Sequencing depths were then accounted for by normalization of raw counts. To identify genes that had significant differences differential expression analysis was performed, and the *p*-values were adjusted. Genes with significant differential expression were identified using statistical cutoffs with a threshold of $P < 0.05$ and log2 fold change (log2FC)≠0. To understand the roles of detected genes their connection to functional categories were identified using GO terms.

5. Result

The results show a variation in total biomass production of the emitters (Figure 4A). Fairytale exhibited lowest total biomass, significantly below Salome *(p=0.0017*), while Luhkas did not differ from either cultivar (Ft–L *p=0.054* and L–S *p =0.35*). The same pattern could be observed in above-ground biomass (*Figure* 5), stem biomass (Figure 7)*,* leaf biomass (Figure 6) and root biomass (*Figure* 8). Fairytale consistently produced significantly less biomass than Salome across all traits which confirms Fairytale as the slow-growing genotype. These contrasts were mirrored in the receiver responses whereas plants exposed to volatiles from Fairytale accumulated less biomass than those exposed to Salome. However, no differences in biomass were observed between SES and SEL nor between SEFt and SEL (Figure 4, Figure 5, Figure 6, Figure 7, Figure 8).

Root elongation followed a similar pattern whereas Fairytale (Ft) again developed significantly shorter (*P=0.007*) root systems than Salome (S) (Figure 11A), which could also be observed in Salome (SEFt) when exposed to volatiles from Fairytale (P=0.0092) (Figure 11B*).* However, neither SES vs. SEL (P=0.07) nor SEL vs. SEFt (P=0.88) differed significantly.

For leaf traits, Fairytale produced markable smaller leaf surface area than both Salome (*P=0.0001*) and Luhkas (*P=0.022*), whereas Salome had the largest leaves overall (Figure 12A). Similarly, the receiver plants showed significant differences between SEFt and SES (P=0.003), with SES displaying the larger leaf area. Exposure to Luhkas again produced no measurable change (Figure 12B).

Plant and stem did not differ significantly among emitter treatments. In contrast, the receiving cultivars showed clear treatment-dependent responses. Salome exposed to volatiles from Luhkas (SEL), grew significantly shorter than Salome exposed to its own cultivar (SES) both in total plant height *(P=0.0066)* and stem height *(P=0.0009)*. Salome exposed to Fairytale (SEFt) consistently showed intermediate values and did not differ significantly from eighter SEL (*P=0.064–0.088*) or SES (*P=0.087–0.204*) (Figure 9B).

Across all biomass-allocation traits, including shoot root ratio (SR/R), leaf-mass fraction (LMF), stem-mass fraction (SMF), root-mass fraction (RMF), and specific leaf area (SLA), no significant differences were detected among emitters or receiver treatments. Similarly, structural traits such as average root diameter were unaffected. These results indicate that VOC-mediated responses altered the magnitude of growth rather than biomass allocation patterns.

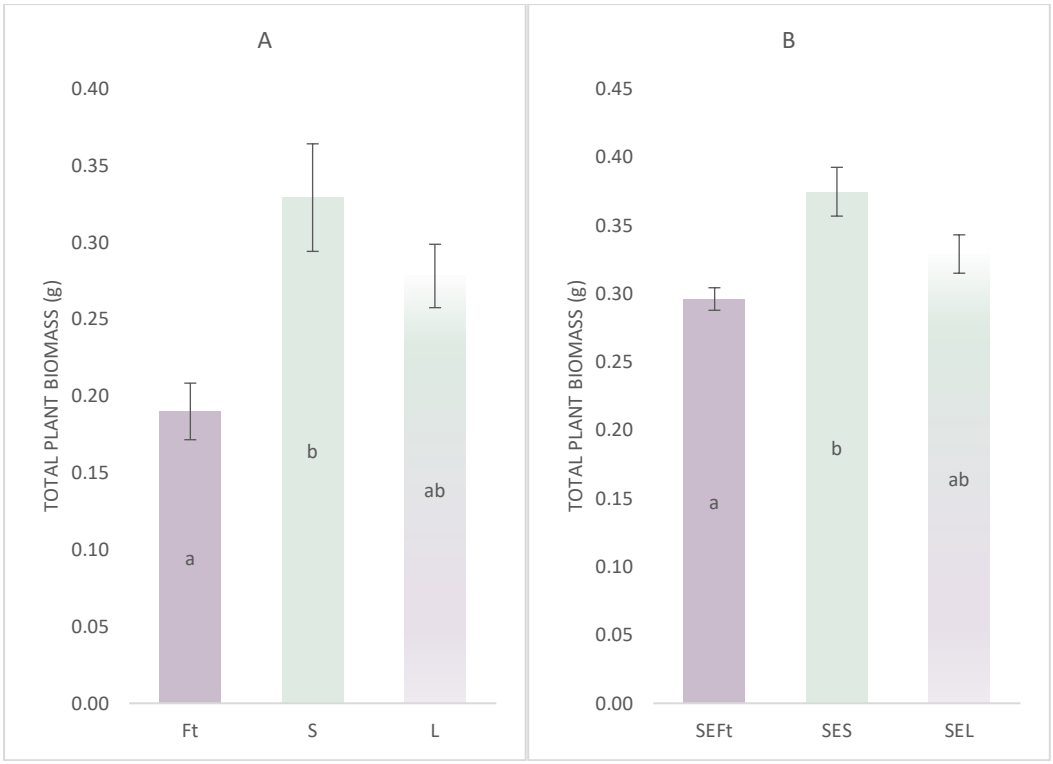

Figure 4. ***Total plant biomass*** *for emitters (A) and receivers (B). Mean value is illustrated on the vertical axis. Error bars show standard error (n=12). The different letters represent significant differences (*P*≤0,05). Columns containing the same letter (a or b) indicates no significant difference (P>0,05).* ***Ft****: Fairytale;* ***S****: Salome;* ***L****: Luhkas;* ***SEFt****: Salome exposed to Fairytale;* ***SES****: Salome exposed to Salome;* ***SEL****: Salome exposed to Luhkas.*

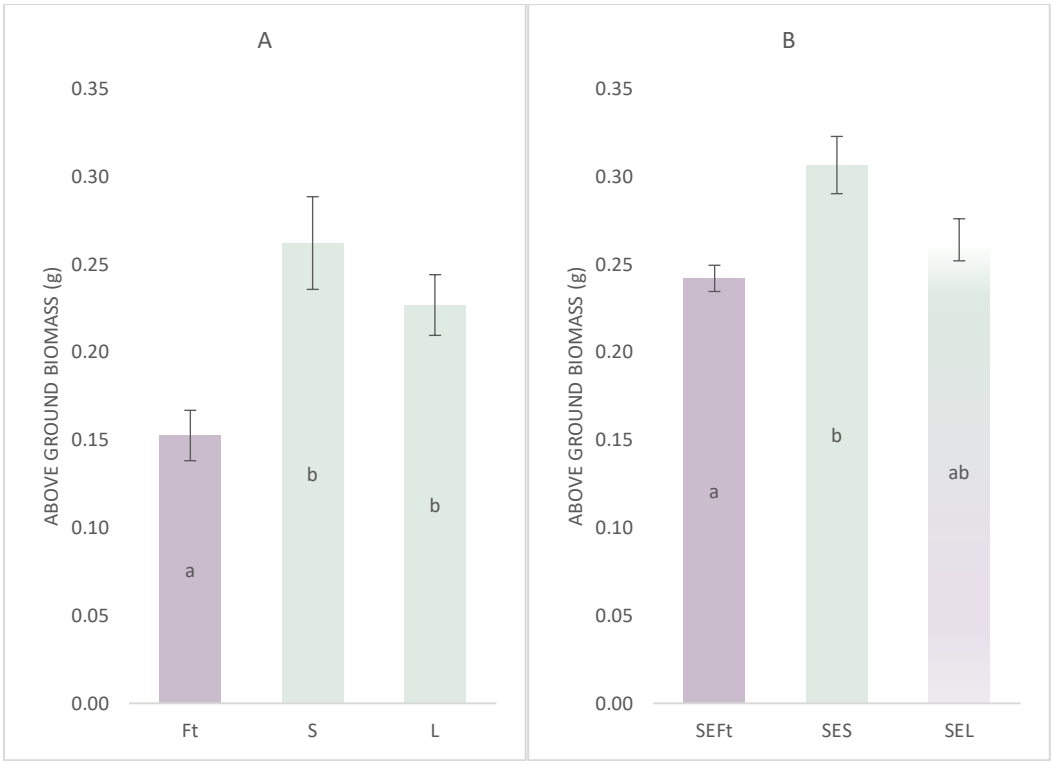

Figure 5: ***Above ground biomass*** *for emitters (A) and receivers (B). Mean value is illustrated on the vertical axis. Error bars show standard error (n=12). The different letters represent significant differences (P≤0,05). Columns containing the same letter (a or b) indicates no significant difference (P>0,05).* ***Ft****: Fairytale;* ***S****: Salome;* ***L****: Luhkas;* ***SEFt****: Salome exposed to Fairytale;* ***SES****: Salome exposed to Salome;* ***SEL****: Salome exposed to Luhkas.*

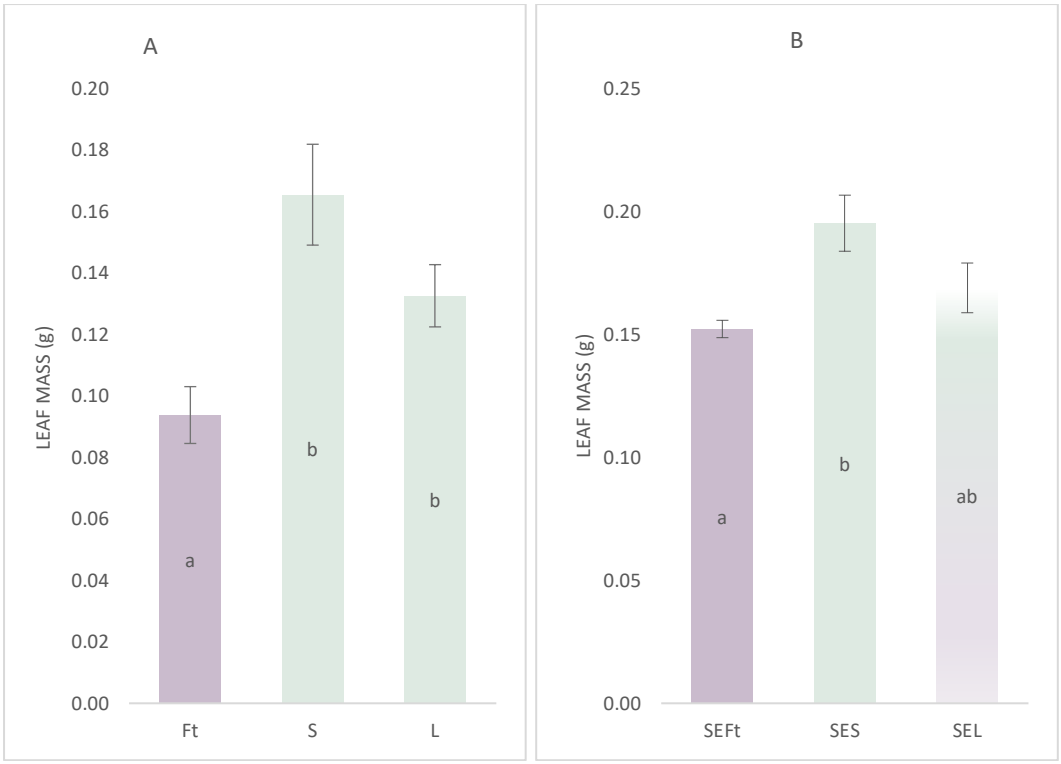

*Figure 6**: Leaf biomass** for emitters (A) and receivers (B). Mean value is illustrated on the vertical axis. Error bars show standard error (n=12). The letters inside the bars (a, b) represent significant differences (P≤0,05). Columns containing the same letter (a or b) indicates no significant difference (P>0,05). **Ft**: Fairytale; **S**: Salome; **L**: Luhkas; **SEFt**: Salome exposed to Fairytale; **SES**: Salome exposed to Salome; **SEL**: Salome exposed to Luhkas.*

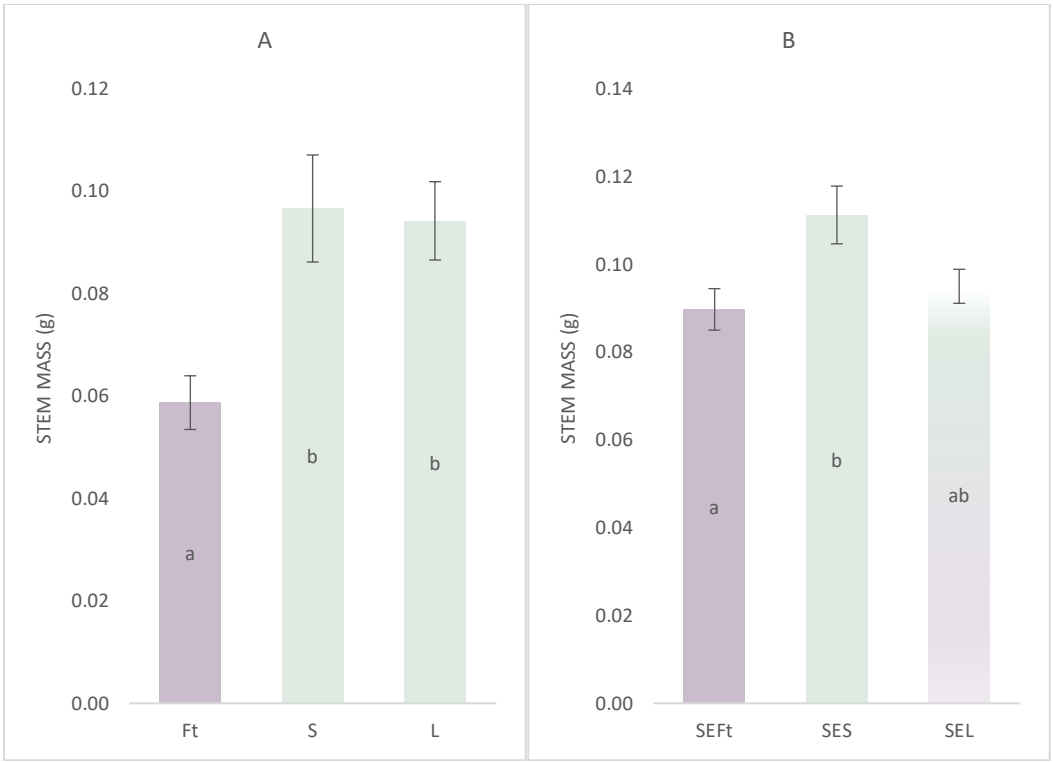

*Figure 7: **Stem biomass** for emitters (A) and receivers (B). Mean value is illustrated on the vertical axis. Error bars show standard error (n=12). The letters inside the bars (a, b) represent significant differences (P≤0,05). Columns containing the same letter (a or b) indicates no significant difference (P>0,05). **Ft**: Fairytale; **S**: Salome; **L**: Luhkas; **SEFt**: Salome exposed to Fairytale; **SES**: Salome exposed to Salome; **SEL**: Salome exposed to Luhkas.*

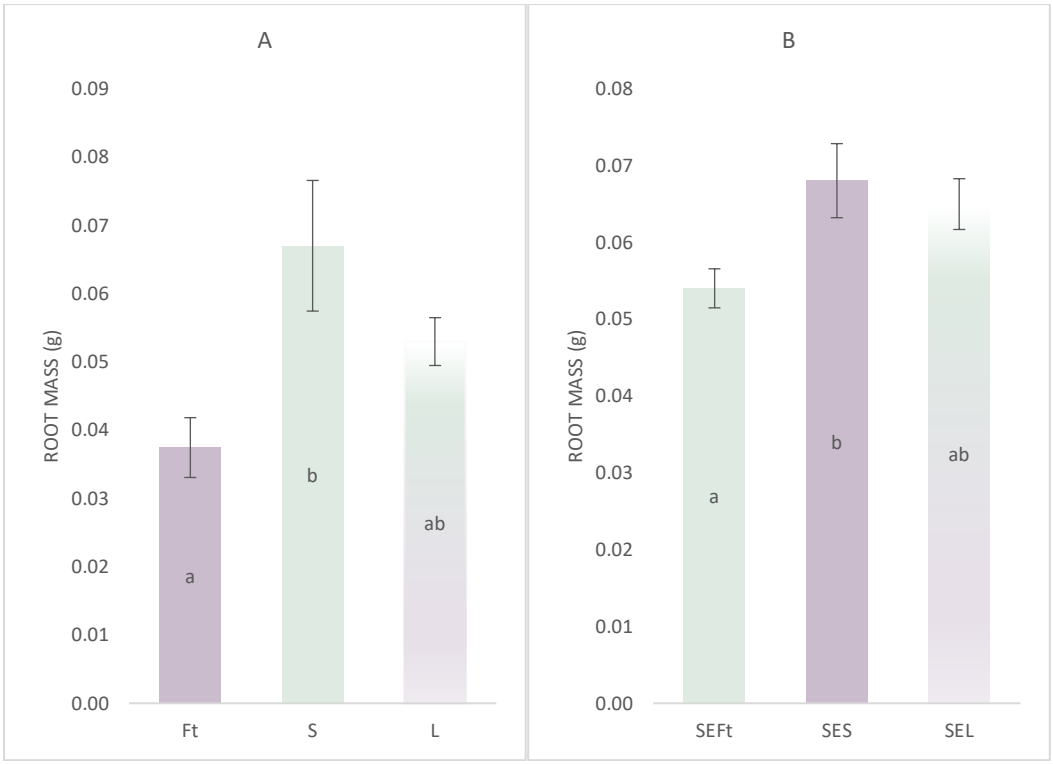

Figure 8: ***Root biomass*** *for emitters (A) and receivers (B). Mean value is illustrated on the vertical axis. Error bars show standard error (n=12). The letters inside the bars (a, b) represent significant differences (P≤0,05). Columns containing the same letter (a or b) indicates no significant difference (P>0,05).* ***Ft****: Fairytale;* ***S****: Salome;* ***L****: Luhkas;* ***SEFt****: Salome exposed to Fairytale;* ***SES****: Salome exposed to Salome;* ***SEL****: Salome exposed to Luhkas.*

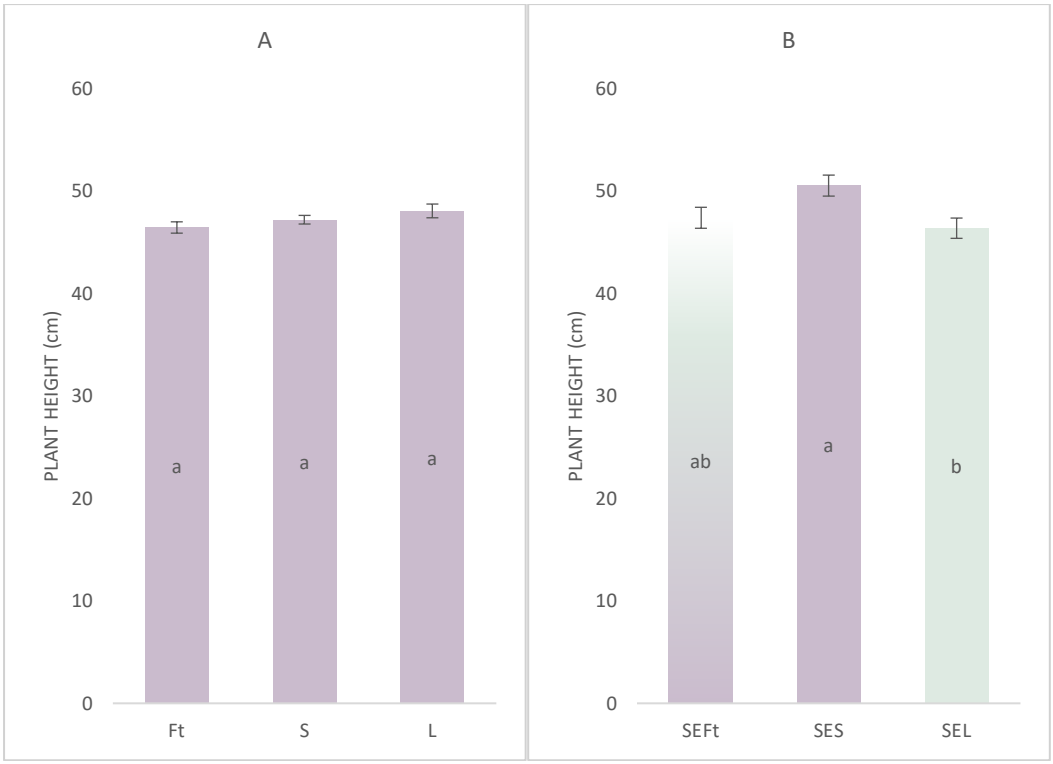

Figure 9: ***Plant height*** *for emitters (A) and receivers (B). Mean value is illustrated on the vertical axis. Error bars show standard error (n=12). The letters inside the bars (a, b) represent significant differences (P≤0,05). Columns containing the same letter (a or b) indicates no significant difference (P>0,05).* ***Ft****: Fairytale;* ***S****: Salome;* ***L****: Luhkas;* ***SEFt****: Salome exposed to Fairytale;* ***SES****: Salome exposed to Salome;* ***SEL****: Salome exposed to Luhkas.*

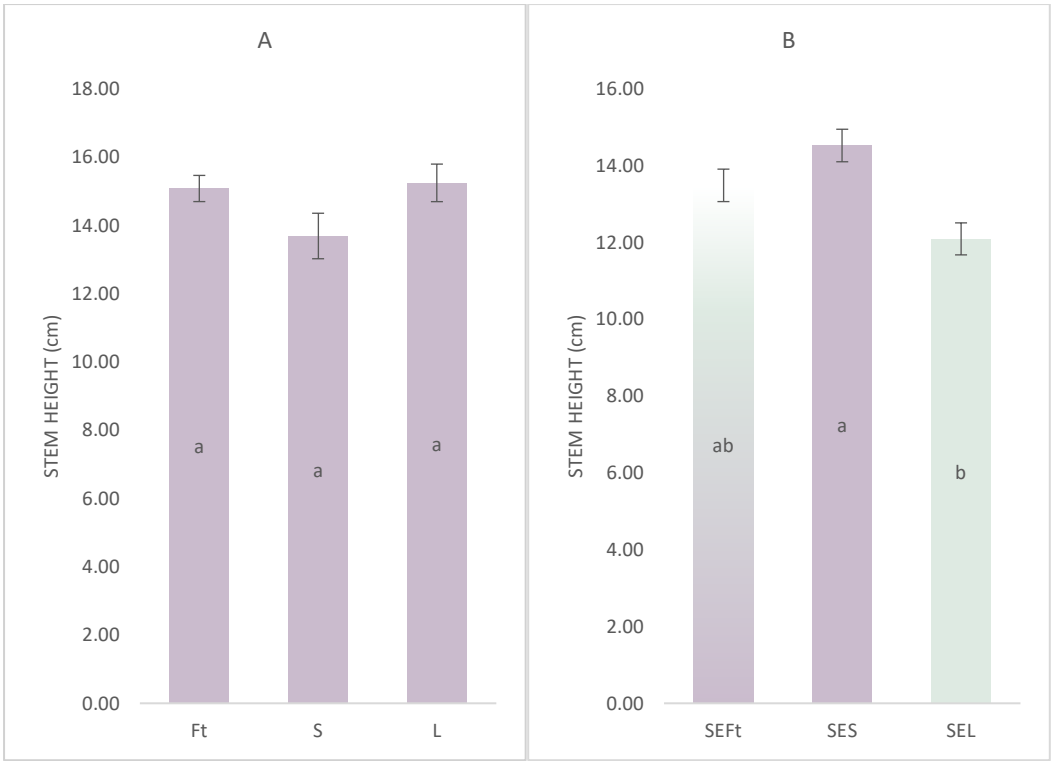

*Figure 10: **Stem height** for emitters (A) and receivers (B). Mean value is illustrated on the vertical axis. Error bars show standard error (n=12). The letters inside the bars (a, b) represent significant differences (P≤0,05). Columns containing the same letter (a or b) indicates no significant difference (P>0,05). **Ft**: Fairytale; **S**: Salome; **L**: Luhkas; **SEFt**: Salome exposed to Fairytale; **SES**: Salome exposed to Salome; **SEL**: Salome exposed to Luhkas.*

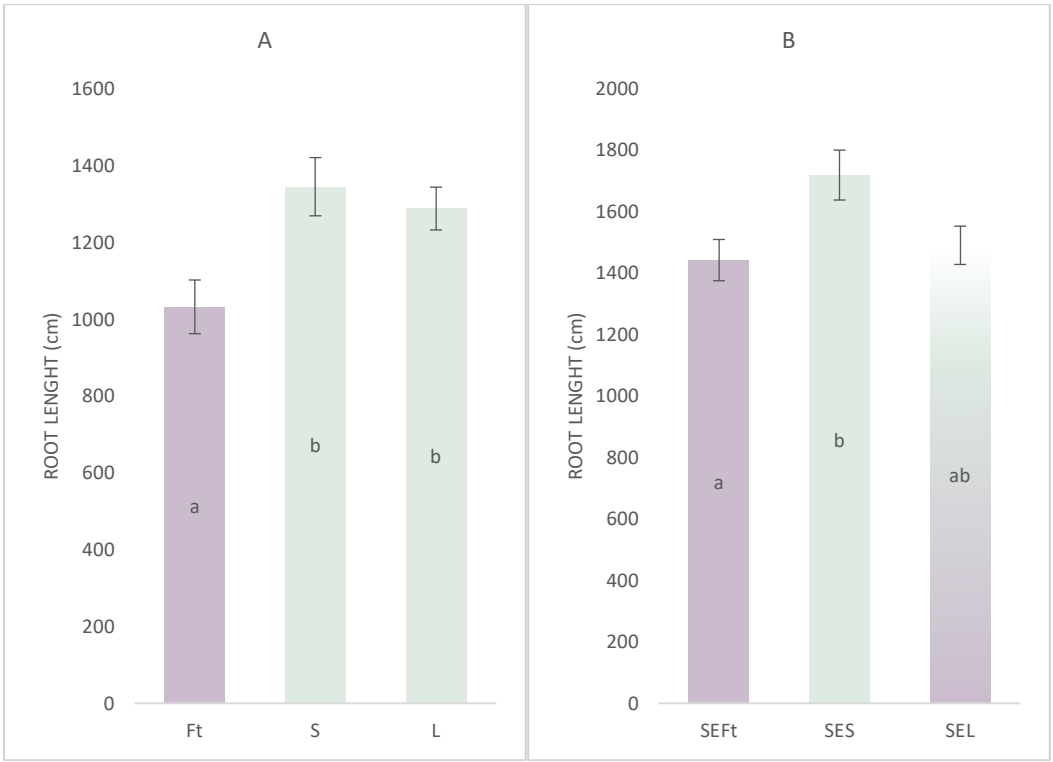

*Figure 11**: Root length** for emitters (A) and receivers (B). Mean value is illustrated on the vertical axis. Error bars show standard error (n=12). The letters inside the bars (a, b) represent significant differences (P≤0,05). Columns containing the same letter (a or b) indicates no significant difference (P>0,05). **Ft**: Fairytale; **S**: Salome; **L**: Luhkas; **SEFt**: Salome exposed to Fairytale; **SES**: Salome exposed to Salome; **SEL**: Salome exposed to Luhkas.*

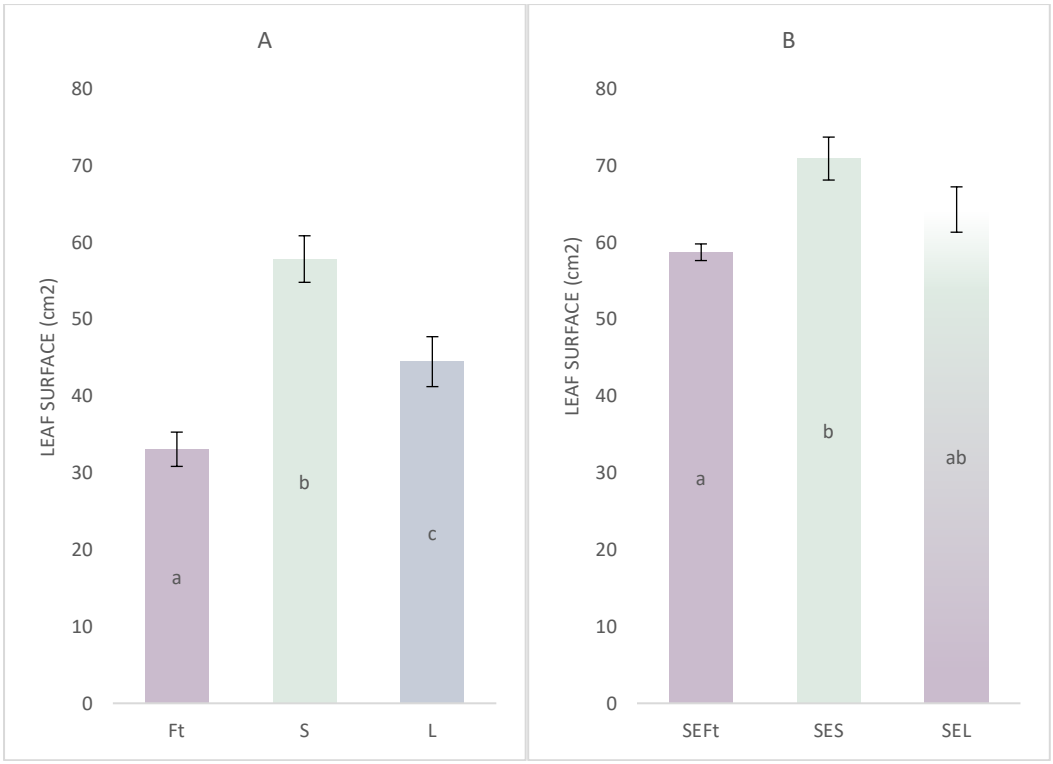

Figure 12: ***Total leaf surface area*** *(cm²) for emitters (A) and receivers (B) is presented on the vertical axis. Error bars indicate the standard error (n = 12). The letters inside the bars (a, b, c) represent significant differences (P≤0.05). Columns containing the same letter (a or b) indicates no significant difference (P>0.05). Ft – Fairytale; S – Salome; L – Luhkas; SEFt – Salome exposed to Fairytale; SES – Salome exposed to Salome; SEL – Salome exposed to Luhkas.*

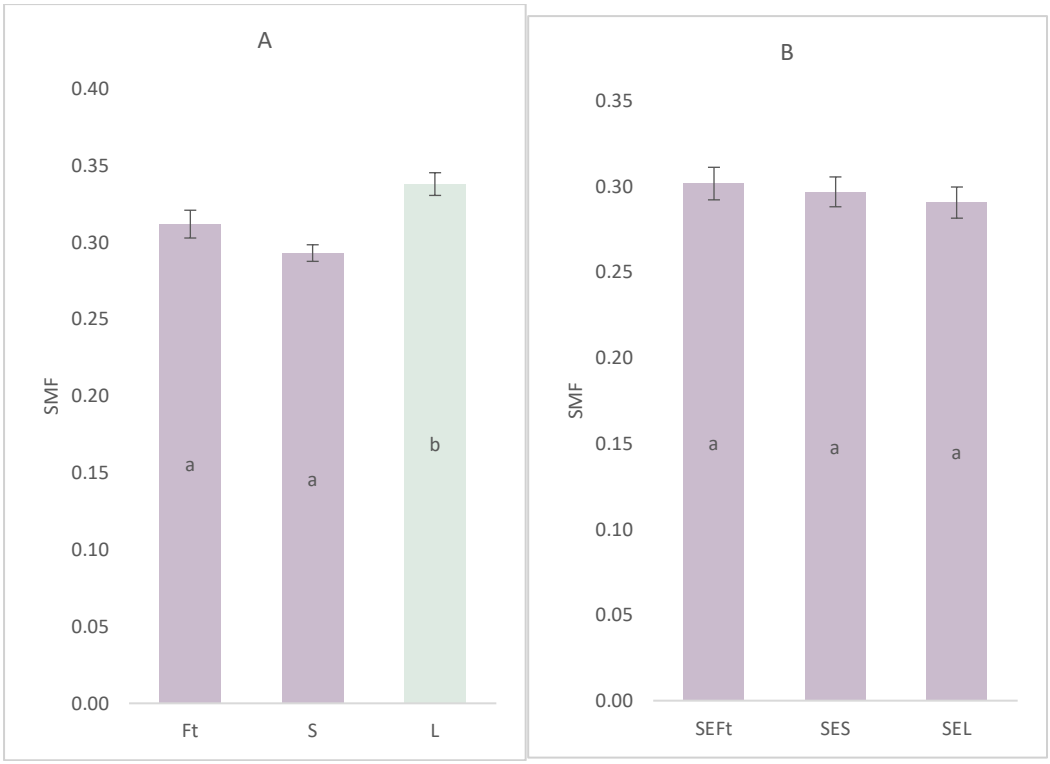

Figure 13: ***Stem Mass Fraction (SMF)*** *for emitters (A) and receivers (B). The mean value is illustrated on the vertical axis. Error bars show standard error (n=12). The letters inside the bars (a, b, c) represent significant differences (P≤0.05). Columns containing the same letter (a or b) indicates no significant difference (P>0.05).* ***Ft****: Fairytale;* ***S****: Salome;* ***L****: Luhkas;* ***SEFt****: Salome exposed to Fairytale;* ***SES****: Salome exposed to Salome;* ***SEL****: Salome exposed to Luhkas.*

Receiver transcriptional responses to VOC-mediated interactions with emitter cultivars

High throughput mRNA libraries were sequenced from two treatments (Salome exposed to Fairytale *Ft* and Salome exposed to Luhkas *L*) and control (Salome exposed to Salome). Differential gene expression analysis detected no significant transcriptional changes in Salome when exposed to Luhkas relative to the control. In contrast exposure to Fairytale induced significant expression changes (adjusted P <0.05) in 14 genes (2 downregulated and 12 upregulated) in comparison to control *(Figure 14)*. Among the upregulated genes in Salome were ubiquitin-specific protease 14 (UBP14), lipid-transfer protein (LTP), two genes from the basic secretory protein family (BSP), protein kinase domain-containing protein (PKD), transcription factor (WRKY), plant cadmium resistance 2 (PCR2), pentatricopeptide repeat protein (PPR) and cotton fiber (CF). Gene ontology (GO) categorization was performed with *mays* as reference for gene annotation. The function of identified genes could then be connected to different kinds of stressors *(Figure 15)*. To further investigate the regulatory potential of these genes, a "guilt-by-association" approach was applied to infer closely connected genes based on co-expression patterns. This analysis identified 481 candidate barley genes (score threshold: 3.6) whose expression profiles were associated with the 14 guide genes (*Figure 16*) identified in the differential gene expression analysis. Subsequent GO categorization of these candidate genes revealed enrichment in functions related to responses to environmental stimuli, including both biotic and abiotic factors (*Figure 17*).

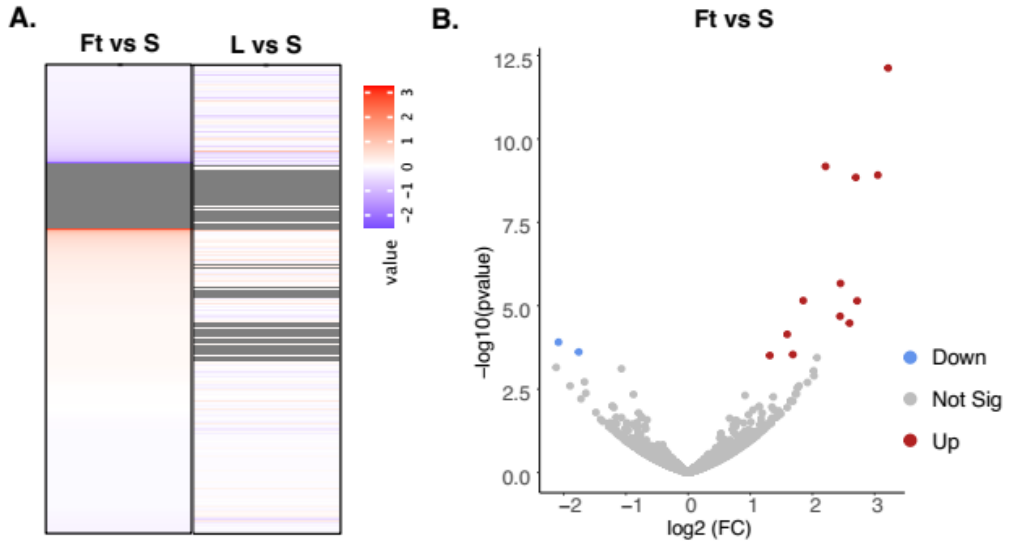

Figure 14: ***Exposure to a different variety induces changes in gene expression. A:*** *Summary of differences in transcriptomic activity when comparing combinations Ft vs S and L vs S.* **B:** *Volcano plot showing the gene expression in Salome exposed to Fairytale (SEFt). Significantly upregulated genes are marked in red while significantly downregulated genes are marked in blue.*
Illustration made by Germán Martinez-Arias.

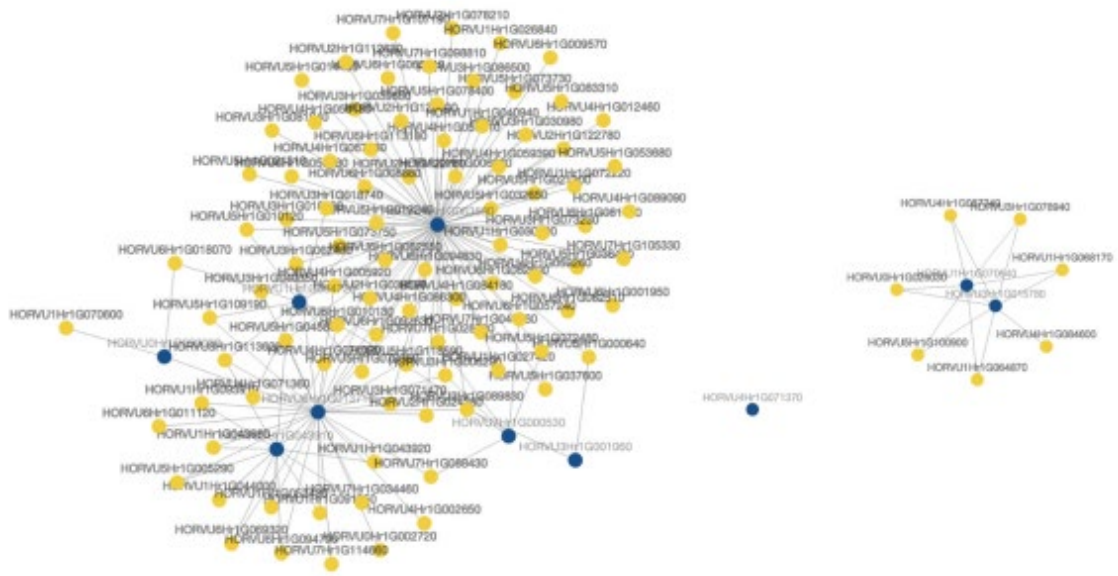

Figure 16: GO enrichment analysis of the genes connected to identified differentially expressed genes in Barley. Annotation to maize has been used as reference.

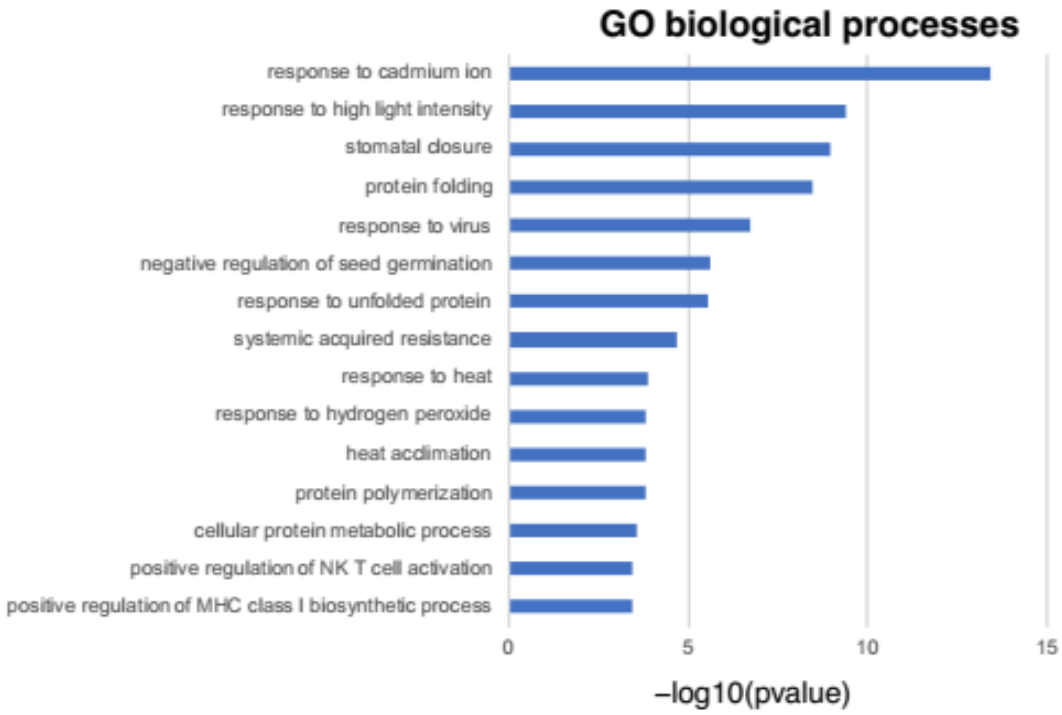

Figure 15: ***Identified differentially expressed genes*** *(blue)* ***and their connection to other genes*** *(yellow) represented in a gene network. Each identified gene has its gene ID above its corresponding dot.*
Illustration made by Germán Martinez-Arias

6. Discussion

Recap of findings

Trait-specific phenotypic differences among cultivars were identified for emitter plants. Among the three cultivars, Salome produced the highest biomass, Fairytale showed the lowest growth and biomass production while Luhkas was intermediate, producing less biomass than Salome but more than Fairytale. Notably, exposure of Salome to Fairytale resulted in a significant reduction in biomass accumulation and was accompanied by differential expression of 14 genes. In contrast, neither Luhkas nor Fairytale showed significant changes in biomass production or transcript profiles when exposed to Fairytale. These findings indicate a receiver specific response in Salome, consistent with capacity to detect and mount physiological and transcriptional adjustment to cues emitted by slower-growing genotype. From a receiver plant's perspective, this could represent a form of adaptive plasticity, adjusting its growth and gene expression in response to perceived competition or environmental cues emitted by neighbouring plants. These results indicates that exposure to volatiles from different genotypes elicits distinct transcriptional responses in Salome, with gene expression changes reflecting the identity of the emitter and involving pathways associated with environmental stress responses.

Similarity increases more in traits with initial greater differences

The findings of this thesis illustrate how growth of a fast-growing cultivar can be reduced following exposure to volatiles from a slower-growing cultivar, thereby increasing phenotypic similarity between neighbours. When attacked by herbivores, emitter plants can respond directly to the cues it emits (Heil & Bueno, 2007; Heil & Karban, 2010). A similar mechanism may also operate under non stressed conditions whereby the emitter reinforces and promotes the development of its own emitter-specific phenotype. When exposed to Fairytale-VOCs, Salome develops a phenotype that is more like Fairytale. Such cases of increased similarity have also been observed in reverse where Fairytale exposed to Salome-VOCs show Salome-specific growth patterns. Just like in this thesis, changes were only detected in biomass production and not in biomass allocation (Åbonde, 2025). However, a different study of cultivars Alva and Kara reports altered biomass allocation with no changes in biomass production (Ninkovic, 2003). Despite different growth parameters affected in the different studies, adaptive similarity could be a common explanation. If non-exposed Alva and Kara produce the same amount of biomass but allocate it differently, increased similarity would not impact biomass production but only its allocation In the Fairytale–Salome comparison, the largest baseline difference lies in biomass production, and this was also the trait most strongly affected by VOC exposure. This pattern suggests that volatile-mediated interactions tend to modify the traits that most strongly differentiate the interacting cultivars.

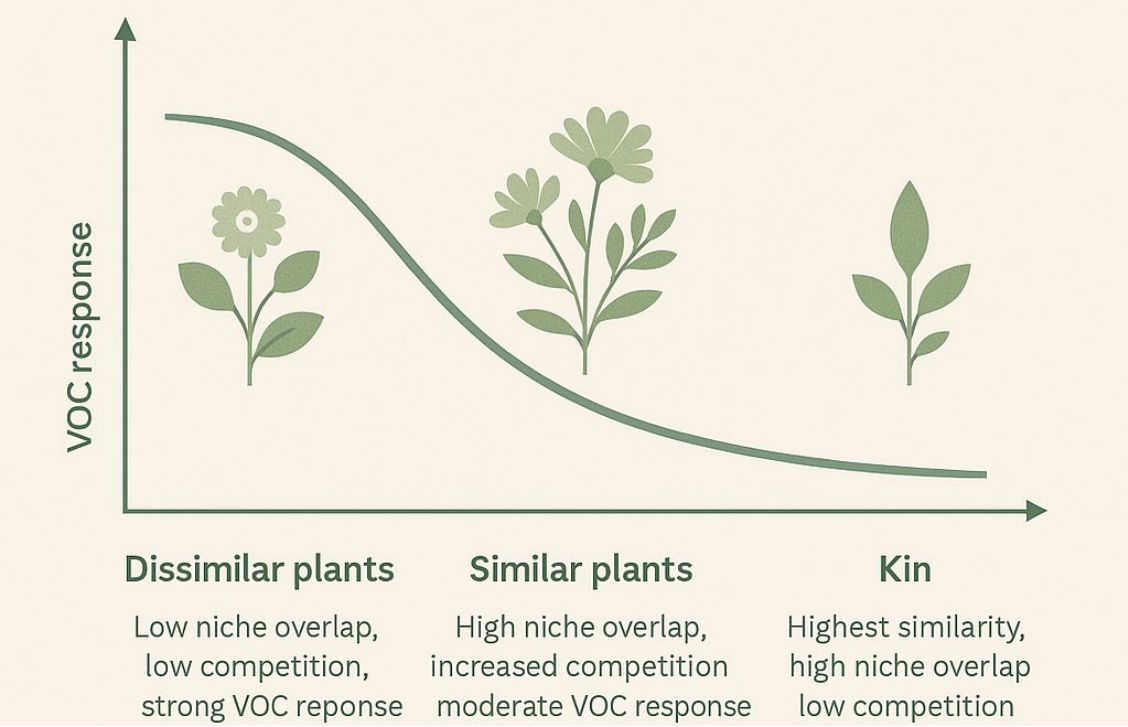

Figure 17: ***Diagram showing the correlation between similarity and level of response to VOCs****.*
VOC Response refers to the level of reaction which includes altered gene expression, growth, defense priming or reallocation of resources. Salome and Fairytale have initially low similarity and therefore Salome show high VOC response by increasing their similarity upon exposure. This results in higher niche overlap and increased competition which could be observed in altered gene expression of Salome exposed to Fairytale. Salome and Luhkas have initially similar phenotypes and moderate to low VOC response. Figure created using AI-generated illustration based on author's conceptual model (Microsoft Copilot, 2025).

VOC responses may rather depend on plant similarity than identity

VOC-exposure triggers diverse pairing-dependent responses and increased phenotypic similarity may represent a common outcome in plant-plant interactions. In some cases, such changes might be subtle and therefore not detected through phenotypic analysis which could explain the lack of significant observations in combinations with Luhkas. Similar alterations have been observed in more than one study (Åbonde, 2025). One experiment report that VOCs from Luhkas have greater impact on Salome than VOCs from Fairytale, and also showed that Salome and Fairytale are more similar to each other in cultivar-specific traits than Salome and Luhkas (Dahlin, 2019). Combinations of Salome and Fairytale have produced convergence in some cases (Åbonde, 2025) but not in others (Dahlin et al., 2020), raising question about the consistency of cultivar specific response to neighbour VOCs. A recurring pattern is that the magnitude of convergence tends to scale with the initial phenotypic divergence between interacting cultivars: pairs that differ more strongly often show larger convergence responses *(Figure 18)*. At the same time, each genotype or cultivar posses a unique volatile profile (Dahlin et al., 2018) meaning that plants can differ chemically even when they appear phenotypically similar.
If there is a correlation between differences in volatile profiles and adaptive similarity, adaptive similarity might occur frequently among non-stressed plants but become less detectable in pairs that already share similar phenotypes.

Mimicry and Defence: The Dual Strategies in Response to Fairytale

Even if the concept of VOCs derived from within-plant-signalling, the capability to sense and respond do volatile cues likely have other evolutionary gains. Plants with the ability to eavesdrop on its neighbours might get a competitive advantage (Heil & Karban, 2010) identified by adverse interactions harming one of the plants, with a negative impact on both plants in the long run (Mooney, 1997). Salome exposed to Fairytale produced less total biomass and since Fairytale is smaller than Salome, this could be a sign of mimicry (Dudley et al., 2013). Since Fairytale is less competitive, Salome may allocate fewer resources to competitive behaviour, which could lead to reduced growth. However, a decreasing resource investment in growth may be accompanied by a corresponding increase in defence. The upregulation of defence-related genes in Salome implies on a competitive strategy rather than adaptation for co-existence. However, in a study where the Fairytale was exposed to the faster growing cultivar Salome, biomass production in Fairytale increased (Åbonde, 2025) indicating mimicry rather than resources being reallocated from growth to defence. Externally Salome adapts strategies for avoiding competition while internally priming for future competition. Another study shows how exposure to Fairytale improves aphid suppression in Salome (Kheam et al., 2023) which could be a result of upregulated defence-genes at a higher trophic level. Priming for competition have also been observed for non-stressed individuals in earlier experiments (Ninkovic et al., 2016). This dualistic approach illustrates the plasticity of plants and how self-preservation is maintained while competition is reduced to a minimum.

No signs of competition despite overlapping niches with Luhkas

Competitive traits are often triggered by stress due to resource limitation. The risk for this is higher for similar cultivars with overlapping niches (Lepik et al., 2012). Luhkas and Salome are initially similar which could naturally increase competition (Li et al., 2018) but in this study Salome shows no significant responses to VOCs from Luhkas. Maybe volatile cues emitted from Luhkas are less distinctive, creating a more passive interaction which together with the lack of resource limitation reduces the need for competitive adaptation. Adaptation for coexistence is usually beneficial for both individuals, therefore a strategy of "wait-and-see" is preferably adopted until perception of a clear competitive signal. Alternatively, the similarity of Salome and Luhkas might be closer to kin recognition in the gradient of similarity *(Figure 18)* which correlates to low VOC responses, that could not be detected in this experiment.

Conclusion

The result of this study illustrates that volatile mediated interactions among barley cultivars can elicit distinct, pairing-dependent effects. By focusing on the cultivar Salome as receiver, exposure to volatiles from slow-growing Fairytale induced a clear reduction in biomass and was accompanied by upregulation of stress- and defence-associated genes, whereas exposure to Luhkas, did not produce detectable phenotypic or transcriptional changes. These findings support the hypothesis that degree of VOC-induced responses is influenced more by the initial dissimilarity between emitter and receiver than by cultivar identity per se. Larger baseline difference were associated with stronger trait convergence, suggesting that VOCs may act as cues for adaptive mimicry, competitive priming or both. This plasticity in response highlights the varying strategies plants employ to optimize coexistence or outcompete neighbours that are context dependent.

Importantly, the results highlight the potential of cultivar mixtures as a tool for enhancing crop resilience and sustainability. By strategically combining cultivars with complementary traits, it may be possible to harness beneficial VOC interactions to improve growth performance, reduce pest susceptibility, and minimize reliance on external inputs. Future research should explore these dynamics in field conditions and across a broader range of cultivars to further elucidate the ecological and agricultural implications of VOC-mediated plant communication.

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7. Appendix

Light Recipe

LED Blue	LED Green	LED Red	LED Far-red	Total	PAR
24,8	34,2	31	4,8	94,8	90,0

Table 2: Properties of light spectra provided for each plant. All values for light spectra are presented in µmol/s/m2. (PAR: Photosynthetically Active Radiation. Proportion usable for photosynthesis)

Fertilizer Recipe

BLOMSTRA	
Nitrogen	4,6%
Phosphor	0,9%
Kalium	3,8%
Sulphur	0,35%
Calcium	0,26%
Magnesium	0,35%
Iron	0,015%
Manganese	0,017%
Boron	0,009%
Zink	0,0026%
Copper	0,0013%
Molybdenium	0,00035%

*Table 3: **Composition of Blomstra Plant nutrition** with pH 3,0; at a 2ml/l dilution and with a density of 1,145 g/cm³ at 20°C.*

Irrigation Schedule

Days after Sowing	Source	06:00-22:00	22:00-06:00
1-6	Water	On: 15 min Off: 15 min	On: 15 min Off: 30 min
7-10	Fertilizer	On: 15 min Off: 15 min	On: 15 min Off: 30 min
11-16	Fertilizer	On: 15 min Off: 30 min	On: 15 min Off: 45 min
17-20	Fertilizer	On: 15 min Off: 45 min	On: 15 min Off: 60 min
21-27	Fertilizer	On: 15 min Off: 60 min	On: 15 min Off: 75 min

Table 4: ***Irrigation Schedule;*** *Programme for the irrigation system*

Mean values of different growth parameters

Biomass

Treatment		Total plant biomass	Above ground biomass	Leaf mass	Stem mass	Root mass
SEFT	Mean Value	0,296003	0,241990	0,152258	0,089732	0,054013
	Standard error	0,008256	0,007467	0,003535	0,004726	0,002539
SEL	Mean Value	0,328948	0,263969	0,168976	0,094993	0,064979
	Standard error	0,017861	0,011977	0,010111	0,003871	0,003305
SES	Mean Value	0,374561	0,306535	0,195286	0,111249	0,068026
	Standard error	0,017861	0,016296	0,011403	0,006575	0,004822
Ft	Mean Value	0,189958	0,152511	0,093800	0,058711	0,037447
	Standard error	0,018410	0,014346	0,009248	0,005242	0,004380
L	Mean Value	0,278122	0,226819	0,132617	0,094203	0,052969
	Standard error	0,020634	0,017283	0,010127	0,007648	0,003504
S	Mean Value	0,329119	0,262119	0,165505	0,096614	0,067000
	Standard error	0,035001	0,026385	0,016409	0,010447	0,009565

Table 5: ***Biomass Parameters;*** *Mean values and standard errors of the different biomass parameters. Total plant biomass (g), Above ground biomass (g), Leaf mass (g), Stem mass (g), Root mass (g).*

Elongation

Treatment		Plant height	Stem Height	Root Length	Leaf surface	Root Diameter
SEFT	Mean Value	47,400000	13,483333	1442,288269	58,712139	0,317908
	Standard error	1,024177423	0,42244478	67,16391771	1,083019395	0,008514
SEL	Mean Value	46,383333	12,090152	1490,444286	64,270850	0,327892
	Standard error	0,990013264	0,41644416	62,37192	2,952065753	0,006937
SES	Mean Value	50,541667	14,520988	1719,048264	70,903208	0,308229
	Standard error	1,024728961	0,4230895	81,26832278	2,796197198	0,009413
Ft	Mean Value	46,452778	15,076923	1032,16783	33,067000	0,306558
	Standard error	0,553055613	0,383250492	69,9077396	2,231241354	0,015576
L	Mean Value	48,070833	15,241667	1288,04415	44,470061	0,314654
	Standard error	0,671099922	0,549787608	55,7427583	3,249948626	0,013829
S	Mean Value	47,212500	13,685897	1344,93812	57,825400	0,325029
	Standard error	0,420548208	0,666209611	75,8044267	3,029934434	0,011845

*Table 6: **Elongation Parameters;** Mean values and standard errors for elongation parameters. Plant height (cm), Stem height (cm), Root length (cm), Leaf surface (cm²), Root diameter (mm).*

Biomass Allocation

Treatment		SR/R	LMF	SMF	RMF	SLA
SEFT	Mean Value	4,608613	0,515446	0,301665	0,182889	387,540132
	Standard error	0,320441	0,006280	0,009491	0,007439	10,16459
SEL	Mean Value	4,129386	0,511419	0,290571	0,198010	384,928767
	Standard error	0,204352	0,013429	0,009093	0,007026	11,74669
SES	Mean Value	4,747268	0,520008	0,296840	0,183153	369,965428
	Standard error	0,410588	0,013978	0,008706	0,011974	13,63225
Ft	Mean Value	4,219089	0,493152	0,311876	0,194971	366,296332
	Standard error	0,202294	0,006543	0,009040	0,007938	16,9076051
L	Mean Value	4,478441	0,476260	0,337943	0,185797	339,009220
	Standard error	0,214950	0,009269	0,007388	0,007624	14,6656567
S	Mean Value	4,119801	0,506941	0,293040	0,200019	370,619423
	Standard error	0,224613	0,011915	0,005399	0,009852	22,7304463

Table 7: ***Biomass Allocation Parameters;*** *Mean values and standard errors of parameters related to biomass allocation; Shoot-root ratio (SR/R), Leaf-mass fraction (LMF), Stem-mass fraction (SMF), Root-mass fraction (RMF) and SLA (Specific Leaf Area).*

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